Vanadyl Sulfate Prevents Fructose-Induced Hyperinsulinemia and Hypertension in Rats

Sanjay Bhanot, John H. McNeill, Michael Bryer-Ash

Abstract To determine whether insulin resistance and hyperinsulinemia are causally related to fructose-induced hypertension, we used vanadyl sulfate, a drug that improves insulin sensitivity in rats. Chronic oral vanadyl treatment was initiated in 6-week-old male Sprague-Dawley rats. One week after vanadyl was started, rats were fed either normal rat chow or a fructose-enriched diet. Plasma glucose and insulin levels and systolic blood pressure were measured weekly for 4 weeks. Fructose feeding induced hyperinsulinemia (fructose-fed, 366.6±8.4 versus control, 276±10.8 pmol/L, P<.001) and increased blood pressure (fructose-fed, 160±3.0 versus control, 124±3.0 mm Hg, P<.001). Vanadyl (0.4 to 0.6 mmol/kg per day) prevented the rise in plasma insulin (treated, 211±2±6.0 pmol/L, P<.001) and blood pressure (treated, 127±3.0 mm Hg, P<.001) in the fructose-fed rats without a change in plasma glucose. No change in blood pressure was seen in the control group. After 4 weeks, euglycemic clamps were performed on 20-hour-fasted, conscious, mobile rats. Low-dose porcine insulin infusion (14 pmol/kg per minute) with concomitant somatostatin infusion resulted in similar steady-state plasma glucose and insulin levels in the various groups. Hepatic glucose production was suppressed and similar among various groups under clamp conditions. Insulin sensitivity index (micromoles of glucose per kilogram per hour per picomole per liter of insulin) was reduced in the fructose-fed rats compared with controls (fructose-fed, 0.9±0.4 versus control, 5.4±1.2, P<.002). Vanadyl caused a marked enhancement in the insulin sensitivity index in the fructose-fed rats (5.3±0.7), without any change in the control group (8.3±1.6, P>.05). Restoration of plasma insulin in the fructose-vanadyl-treated rats to pretreatment levels (daily subcutaneous Ultralente insulin, 14,000 pmol/kg per day) reversed the effects of vanadyl on blood pressure (vanadyl-treated plus insulin, 170±9.8 mm Hg), without a change in plasma glucose. We conclude that hyperinsulinemia, secondary to insulin resistance, may contribute to the development of fructose-induced hypertension and that vanadyl sulfate prevents fructose-induced increases in plasma insulin levels and blood pressure.

Key Words • insulin resistance • hyperinsulinemia • hypertension, essential • vanadyl sulfate

Insulin resistance and hyperinsulinemia are common findings in patients with essential hypertension.1 These defects in glucose metabolism are associated with a high atherogenic risk profile, and recent evidence suggests that they may play a role in the development of hypertension, dyslipidemia, and atherosclerosis.2 3 Insulin resistance has also been documented in several models of experimental hypertension, including the fructose hypertensive rat.4 The fructose rat model represents an acquired form of systolic hypertension, in which the rise in blood pressure (BP) is not genetically determined but is diet induced.5 Although the precise mechanism by which hypertension develops in fructose-fed rats has not been defined, it has been proposed that the rise in BP is secondary to the development of insulin resistance and hyperinsulinemia.6 7 If the increase in BP in fructose-fed rats is secondary to the insulin resistance and hyperinsulinemia that occurs on feeding fructose, then a drug intervention that reverses these effects should also attenuate the hypertension. To examine this proposition, we attempted to improve insulin sensitivity in the fructose-fed rat by using vanadyl sulfate, a drug known to exhibit insulin-mimetic effects.8 9 Vanadyl is the (+IV) form of the trace element vanadium and has been shown to lower plasma glucose levels in diabetic rats without causing an increase in plasma insulin concentration.9 It has also been demonstrated that vanadyl decreases insulin levels in nondiabetic rats without affecting plasma glucose levels, suggesting that it replaces or potentiates the action of endogenous insulin.8 10 The objective of the present study was to examine the relation between insulin resistance, hyperinsulinemia, and BP in fructose hypertensive rats. Therefore, vanadyl sulfate was administered to fructose-fed rats, and the effects of the drug on insulin sensitivity, plasma insulin levels, and systolic BP were studied.

Methods

General Protocol
Male Sprague-Dawley rats were procured locally (body weight, 180 to 200 g, 6 weeks of age). The animals were randomly assigned to four experimental groups: control (C, n=8), control vanadyl-treated (V, n=12), fructose-fed (F, n=9), and fructose-vanadyl-treated (FV, n=15). At week 6 (weeks denote the age of the animals) BP, plasma glucose, and plasma insulin (5-hour fasted) were measured in all groups. Subsequently, chronic vanadyl sulfate treatment (VOSO₄-nH₂O, Fisher Scientific) was initiated in the V and FV groups. Rats received vanadyl at a concentration of 0.75 mg/mL ad libitum in the drinking water. This concentration was chosen because previous studies in our laboratory showed that it decreased insulin levels in nondiabetic rats without altering plasma glucose levels.9 One week after initiation of vanadyl treatment, the animals in the F and FV groups were...
started on a 66% fructose diet (Teklad Premier Laboratory Diets). The electrolyte content of the fructose diet was similar to that of the standard rat chow (standard chow: 4.9 g/kg sodium and 11.8 g/kg potassium; fructose diet: 4.2 g/kg sodium and 10.8 g/kg potassium). Systolic BP, plasma insulin (5-hour fasted), and plasma glucose were measured each week for the next 4 weeks. In addition, food intake, fluid intake, and body weights of the animals were recorded every week. At termination, insulin sensitivity was assessed in conscious rats by the euglycemic, hyperinsulinemic clamp technique. All experimental procedures were approved by the University of British Columbia Animal Care Committee.

Blood Pressure Measurement

Indirect systolic BP was measured in conscious rats using the indirect tail-cuff method without external preheating.11 The animals were preconditioned to the experimental procedure before actual measurements were conducted. The equipment used included a BP sensor/cuff, a BP amplifier, and an analog-to-digital recorder and printer (model 179 semiautomatic BP analyzer, IITC Inc). In this method, the reappearance of pulsations (on gradual deflation of the BP cuff) are detected by a photoelectric sensor and are amplified and recorded digitally as the systolic BP. An average of five such readings was taken as the individual systolic BP. The major advantage of this method is that the recordings are carried out at a temperature of 26°C to 27°C, thus eliminating the heat stress typical of other methods. In a preliminary study we validated the readings obtained by this method by comparison with those obtained by direct intra-arterial cannulation. Recorded pressures were similar (within 5 mm Hg) to those obtained by other laboratories.6,7

Euglycemic Hyperinsulinemic Clamp Technique

Rats were conditioned to tail restraint by a modification of the approach of Buchanan et al.12 In brief, the tail was passed through a hole (approximately 1.5 cm in diameter) in the cage, after which it was immobilized at a point halfway along its length by passing it through a soft cork and taping it distally. The rats had free access to food and water and were conditioned for increasing periods of time (30 minutes to 2 hours, three times a day) over 3 days before the clamp study. The rats were weighed daily, weight gain was comparable to that observed during the preconditioning period. The rats were fasted overnight (20 hours) before the clamp studies. Four hours before the start of insulin infusions, each animal was placed in a specially designed foam rubber jacket, which allowed free movement of all four limbs and forward vision. Subsequently, the rat was placed on a board with a belt positioning system that allowed it to be immobilized in the left or right lateral and supine positions. Lidocaine (1%) was then infiltrated into the tissue on the ventral aspect of the tail. A 0.5-cm incision was made in the tail, and the tail artery was cannulated with fine-bore polyethylene tubing (PE-10) and flushed with 50 U/mL heparin in 0.9% saline. The tail vein was then cannulated percutaneously with a 24-gauge Intracath (Jelco) attached to PE-50 tubing. The animal was returned to the cage and allowed to recover with free access to water. Fifteen minutes before the start of the clamp studies, an infusion of d-[3-3H]glucose was initiated by a 100-fold square-wave bolus over 1 minute, followed by a continuous infusion of 0.10 μCi/min from 0 to 120 minutes. This was done for isotope determination of glucose turnover as described by Steele.13 During the first 15 minutes of the clamp, baseline plasma glucose measurements were obtained. At 0 minutes, an infusion of insulin at a rate of 14 pmol/kg per minute was begun. This was preceded by a loading dose of 3x, 2x, and 1x (the steady-state infusion rate) for 1 minute each. Somatostatin was infused at a rate of 920 pmol/kg per minute throughout the clamp study (from 0 to 120 minutes) to suppress endogenous insulin production. During the clamps, 20% D-glucose was infused as needed to maintain plasma glucose at the preinfusion level. Thirty microliters of arterial blood was sampled at 5-minute intervals for determination of plasma glucose. In addition, 50 μL of blood was withdrawn immediately after surgery and at 15, 30, 60, and 120 minutes after surgery for determination of catecholamine levels. During the last 30 minutes of the clamp (at 100, 110, and 120 minutes), 200 μL of blood was withdrawn for measurement of steady-state plasma insulin levels and tracer dilution. After this, the animals were killed by an intravenous injection of pentobarbital (250 mg/kg).

Biochemical Measurements

Plasma glucose levels were measured by the glucose oxidase method in a YSI 23A glucose analyzer (Yellow Springs Instrument Co). Plasma insulin levels were assayed by a double-antibody radioimmunoassay technique using human insulin standards (ICN Biomedicals Inc). For determination of d-[3-3H]glucose concentrations, serum was diluted 1:4 with water and then added to an equal volume of perchloric acid (final concentration, 2.5%). Proteins were precipitated by centrifugation at 2000g for 10 minutes. Aliquots of supernatant were dehydrated for 6 hours at 55°C and counted in a β-scintillation counter. Plasma catecholamines were measured by a radioenzymatic method (Amersham Inc).

Statistical Analyses

All data are presented as mean±SEM and were analyzed by MANOVA followed by a Neuman-Keuls Test, using the Number Cruncher Statistical System (NCSS). Changes within each group over time were analyzed by one-way ANOVA followed by a Neuman-Keuls Test. A probability level of <.05 was taken to indicate a significant difference between means.

Results

Blood Pressure, Plasma Insulin, and Plasma Glucose

Fructose feeding caused an increase in 5-hour fasting plasma insulin levels that persisted throughout the study (mean baseline insulin, 229.8±19.8 versus mean at 9 to 11 weeks, 366±8.4 pmol/L; Figure, panel A). Vanadyl sulfate (0.4 to 0.6 mmol/kg per day) completely prevented the rise in plasma insulin in the FV rats (mean baseline insulin, 253.2±14.4 versus mean at 9 to 11 weeks, 211.2±6.0 pmol/L; P<.05). Vanadyl also caused a modest decrease in insulin levels in the V group (baseline, 240.6±15 versus mean at 9 to 11 weeks, 181.2±18.6 pmol/L, P<.05). As illustrated in panel B of the Figure, BP in the F rats increased from 124±3.0 to 160±3.0 mm Hg (P<.001). This increase in BP was evident 2 weeks after the fructose diet was started and persisted throughout the study. In contrast, BP did not rise in the FV rats (mean at baseline, 131±3.0 versus mean at 9 to 11 weeks, 127±3.0 mm Hg, P>.05). No changes in BP were seen in the V group (baseline, 125±5.0 versus mean at 9 to 11 weeks, 126±4.0 mm Hg, P>.05).

To further examine this issue, we administered exogenous insulin (daily subcutaneous Ultralente insulin, 14 000 pmol/kg per day, Eli Lilly) to V and F rats (n=5 in each group) for the next 3 weeks. This was done in an attempt to restore the plasma insulin levels in the FV rats to those seen in the F group and then observe the resultant changes in BP. Restoration of plasma insulin in the FV rats (vanadyl with insulin, 339.6±19.8 versus vanadyl without insulin, 213.6±34.8 pmol/L) reversed the effects of
vanadyl sulfate and caused a corresponding increase in BP (vanadyl with insulin, 170 ± 10 versus vanadyl without insulin, 121 ± 3.0 mm Hg, *P < .001). This increase in BP was independent of changes in body weight (vanadyl with insulin, 353 ± 8 versus vanadyl without insulin, 348 ± 7 g, *P > .05). However, no change in BP was seen in the V rats (vanadyl with insulin, 122 ± 5.0 versus vanadyl without insulin, 119 ± 4.0 mm Hg, *P > .05). In addition, no change in 5-hour fasting plasma glucose was observed after the administration of exogenous insulin in either the V or FV rats.

Fructose feeding did not cause any change in food intake, fluid intake, or body weight compared with the untreated controls (C). Vanadyl treatment resulted in a reduction in weight gain in both the V and FV rats (Table 1). The average plasma glucose values in the various groups ranged from 6.3 to 7.8 mmol/L. Five-hour fasting glucose in all groups remained normal (<8.0 mmol/L), and no changes in plasma glucose were observed after vanadyl treatment in either the V or F rats.

### Euglycemic Clamp Studies

During the 3-day conditioning period, weight gain in the rats was normal and similar to that seen during the previous weeks (data not shown), and none of the rats lost weight. Steady-state plasma glucose levels were similar in the four experimental groups (Table 2) and were well matched to their corresponding basal concentrations. Mean plasma insulin during the final 30 minutes of the clamp were also similar in all four groups. Hepatic glucose production was completely and similarly suppressed in all groups. Negative values were obtained for hepatic glucose production because cold glucose infusate was not "spiked" with [3-3H]glucose during these studies. For each experimental group, insulin sensitivity index was calculated by dividing the rate of glucose appearance (Rg, micromoles of glucose per kilogram per hour) by the steady-state plasma insulin level (picomoles per liter of insulin). As is evident from Table 2, the F rats were severely insulin resistant compared with C rats (insulin sensitivity index, 0.9 ± 0.4 versus 5.4 ± 1.2). Vanadyl treatment caused a marked enhancement in insulin sensitivity index in the FV rats and restored their insulin sensitivity to control levels (5.3 ± 0.7, *P < .002). Plasma catecholamine levels were calculated by taking the mean of values obtained 60 and 120 minutes after surgery, because the levels in all groups fell within the first 30 minutes and remained unchanged thereafter. Catecholamine levels in the FV rats did not change compared with those seen in the F rats (FV, 1473 ± 109 versus F, 1044 ± 69 pg/mL, *P > .05), suggesting that the antihypertensive effects of vanadyl are independent of any change in sympathetic activity. In addition, there was no difference in catecholamine levels between the C and V groups (V, 915 ± 228 versus C, 1478 ± 493 pg/mL, *P > .05), and the values among all four groups were similar at all time points after surgery.

### Discussion

Results from the present study confirm previous reports that feeding otherwise healthy rats a fructose
The fructose diet results in insulin resistance, hyperinsulinemia, and hypertension. The fructose diet (66% fructose, 12% fat, and 22% protein) was specially prepared such that it had an electrolyte, protein, and fat content comparable to the standard rat chow. Therefore, the fructose-induced hypertension was not secondary to changes in dietary sodium intake. It has been reported that the fructose-induced increase in BP is not accompanied by any change in plasma renin activity or angiotensin levels, although the exact role of the renin-angiotensin system in this model of experimental hypertension is still unknown. Evidence suggests that fructose feeding leads to insulin resistance and a compensatory hyperinsulinemic response that in turn may lead to volume overload and hypertension. This notion is supported by studies demonstrating that insulin promotes renal sodium absorption in a variety of species. Hyperinsulinemia can stimulate many hypertensigenic mechanisms, such as activation of the sympathetic nervous system, increase in renal sodium and water reabsorption, and proliferation of vascular smooth muscle tissue (for a review, see Reference 1). However, mechanisms such as increased Na⁺-H⁺ antiporter activity and increased sodium-calcium cellular overload are also worthy of investigation.

If fructose-induced hypertension were secondary to an increase in plasma insulin levels, then a decrease in insulin levels should have prevented the rise in BP. Our results are consistent with this hypothesis, because vanadyl sulfate improved insulin sensitivity and attenuated the increase in both plasma insulin and BP. Vanadyl sulfate treatment did not alter plasma catecholamine levels, suggesting that it lowered BP without any change in sympathetic activity. It can be argued that vanadyl may also affect factors other than insulin; ie, it may decrease BP independent of its effect on insulin action. Vanadyl is a very poor inhibitor of cellular enzyme systems, and we are not aware of any study that indicates any direct antihypertensive effect of vanadyl in vivo at the concentrations used in the present study. Furthermore, restoration of plasma insulin in the FV rats caused a corresponding increase in BP. Reversal of the effects of vanadyl on BP after raising insulin levels to those observed in the F group was independent of changes in body weight, which strengthens the contention that hyperinsulinemia contributes to the genesis of fructose-induced hypertension. Additional support for this hypothesis comes from studies demonstrating that exercise training (which improved insulin sensitivity and decreased insulin levels) and somatostatin administration (which decreased hyperinsulinemia) attenuated the fructose-induced rise in BP. Also, administration of clonidine to fructose-fed rats inhibited the increase in BP but did not improve the associated metabolic defects. This suggests that the defects in carbohydrate metabolism are not secondary to an increase in sympathetic activity.

Interestingly, a modest decrease in plasma insulin levels in the V rats did not cause a decrease in BP, nor did exogenous insulin treatment increase BP. The question arises as to why hyperinsulinemia causes hypertension in insulin-resistant rats without doing so in insulin-sensitive control animals. One possibility is that insulin-sensitive tissues would increase glucose utilization (in response to the increase in insulin levels), which may initiate local autoregulatory vasodilator reflexes to increase local blood flow. By contrast, insulin resistance could prevent such vasodilator responses and thereby result in increased vascular resistance.

In view of reported alterations in glucose metabolism induced by general anesthesia, clamps studies were performed in conscious rats. Buchanan et al reported that catecholamine concentrations returned to normal within 4 hours of cannulation (of the tail artery and vein) in animals preconditioned to partial restraint by the tail. In a modification of his method, we have observed that catecholamine concentrations return to normal within 30 minutes of line placement in both Sprague-Dawley and spontaneously hypertensive rats (unpublished observations). Somatostatin was used to suppress endogenous insulin secretion during the clamp studies, because its effects on glucose clearance are less than those of other agents that have been used to suppress insulin release. The use of somatostatin during clamp studies is based on the assumption that it has no direct effect on tissue glucose metabolism. Although it has been reported that somatostatin causes a small increase in glucose clearance in dogs, Baron et al did not document such an effect during human clamp studies. Furthermore, Buchanan et al reported that during low-dose insulin infusion in rats, somatostatin had no effect on glucose clearance after basal

### Table 2. Results of Glucose Clamp Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>C (n=4)</th>
<th>F (n=4)</th>
<th>V (n=4)</th>
<th>FV (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose (20-hour fasted), mmol/L</td>
<td>3.6±0.0</td>
<td>4.2±0.1*</td>
<td>3.2±0.1</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td>Basal insulin (20-hour fasted), pmol/L</td>
<td>189±58</td>
<td>289±96</td>
<td>76±44</td>
<td>178±29</td>
</tr>
<tr>
<td>Clamp glucose, mmol/L</td>
<td>3.4±0.3</td>
<td>4.3±0.3</td>
<td>3.6±0.1</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>Clamp insulin, pmol/L</td>
<td>475±53</td>
<td>498±104</td>
<td>351±72</td>
<td>389±42</td>
</tr>
<tr>
<td>Clamp Rgl, (mmol/kg)/h</td>
<td>1.4±0</td>
<td>0.8±0.01*</td>
<td>1.8±0.2</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Clamp HGO, (mmol/kg)/h</td>
<td>-1.1±0.4</td>
<td>0.3±0.2</td>
<td>-0.8±0.1</td>
<td>-0.1±0.6</td>
</tr>
<tr>
<td>ISI, mmol/kg/h/pmol/L</td>
<td>5.4±1.2</td>
<td>0.9±0.4*</td>
<td>8.3±1.6</td>
<td>5.3±0.7†</td>
</tr>
</tbody>
</table>

C indicates control; F, fructose-fed rats; V, control vanadyl-treated rats; FV, fructose-vanadyl-treated rats; Rgl, peripheral glucose disposal; HGO, hepatic glucose output; and ISI, Insulin sensitivity index. Values are mean±SEM.

*P<.05 vs C.
†P<.05 vs F.
insulinemia was established. Although somatostatin has been shown to alter levels of plasma glucagon and growth hormone, these findings suggest that the effects of somatostatin on glucose clearance may occur only in the face of low insulin levels or that a difference may exist in the effects of somatostatin on glucose clearance between different species. If glucagon and growth hormone play a different role in glucose metabolism in C, F, and FV groups, the use of somatostatin could have influenced our results. However, looking at the magnitude of the differences in insulin sensitivity among the groups studied, it is perhaps reasonable to conclude that the effects of somatostatin cannot fully account for the observed differences in insulin sensitivity.

Vanadyl treatment resulted in a marked enhancement of insulin sensitivity in the FV rats and restored their insulin sensitivity to control values. However, this was accompanied by a decrease in body weight in the FV group compared with the F group. It therefore may be argued that the improvement in insulin sensitivity in the FV group may be secondary to their lower body weight rather than due to a direct effect of vanadyl itself. Although we do not have an unequivocal answer to this question, we have considered several possibilities. The V rats also showed a similar decrease in body weight (compared with the untreated C group), yet their insulin sensitivity remained unchanged. If a decrease in body weight was the major factor causing an improvement in insulin sensitivity, then there should have been a corresponding increase in insulin sensitivity in the V group, which was not observed. Furthermore, we have expressed insulin sensitivity as the insulin sensitivity index,24 which accounts for changes in body weight that may otherwise confound the results. What is even more important is that the effects of vanadyl on plasma insulin and BP were also observed from weeks 9 to 11, when body weight in the F and FV groups was similar. Finally, reversal of the effects of vanadyl on BP after administration of exogenous insulin was also independent of change in body weight.

In summary, vanadyl sulfate prevents the fructose-induced increase in plasma insulin and BP. The effects of vanadyl on BP can be reversed by restoring plasma insulin levels in the vanadyl-treated rats to pretreatment levels. This suggests that hyperinsulinemia may contribute to the development of high BP in this experimental model or that if hyperinsulinemia and hypertension are not causally related, the underlying mechanism is closely associated with the expression of both disorders. Further studies are needed to examine the role of specific organ systems (sympathetic, renal) in the genesis of fructose hypertension and to determine whether their contribution is primary or secondary to hyperinsulinemia.

Acknowledgments

Supported by funds from the Heart and Stroke Foundation of B.C. and Yukon. S.B. is a Medical Research Council Fellow. M.B.-A. is the recipient of a scholarship and research grant from the B.C. Health Research Foundation.

References


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Hypertension. 1994;23:308-312
doi: 10.1161/01.HYP.23.3.308

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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